Genotype to phenotype

09.01.14

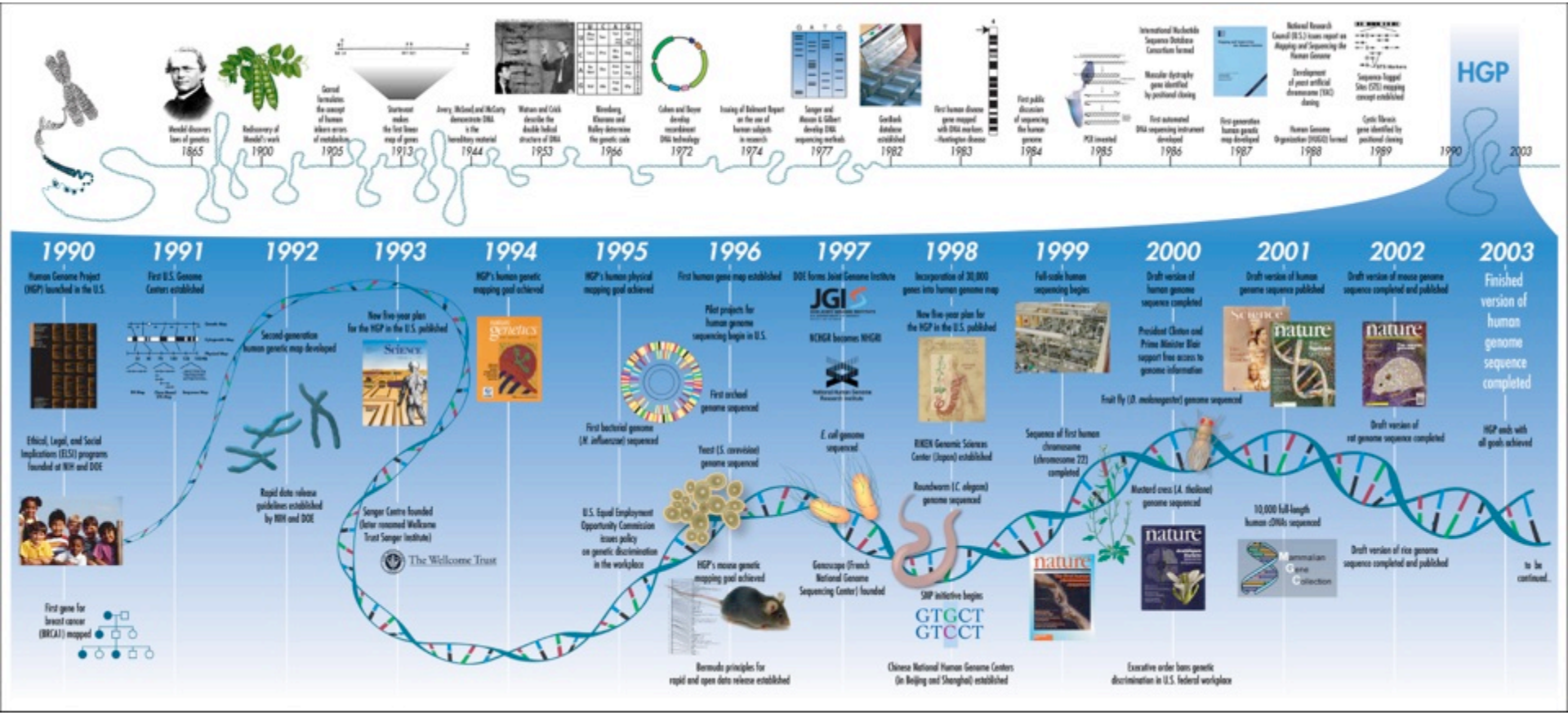
Valborg Gudmundsdottir

Outline for the afternoon

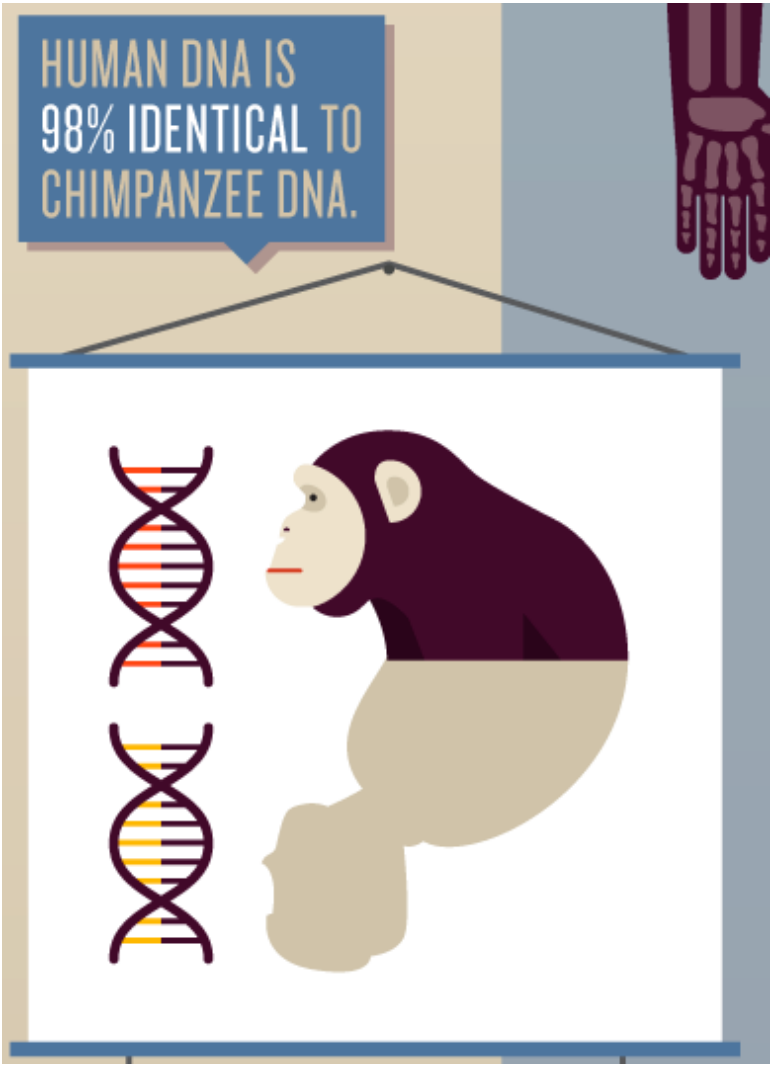
- The human genome
- Genomic variation
- Genotypes, homoyzgotes and heterozygotes
  - *Short exercise*
- Genotyping
- Phenotypes
- Associating genotype with phenotype, genome-wide associated studies (GWAS)
- Genotype to phenotype predictions
  - *Main exercise*

THE HUMAN GENOME

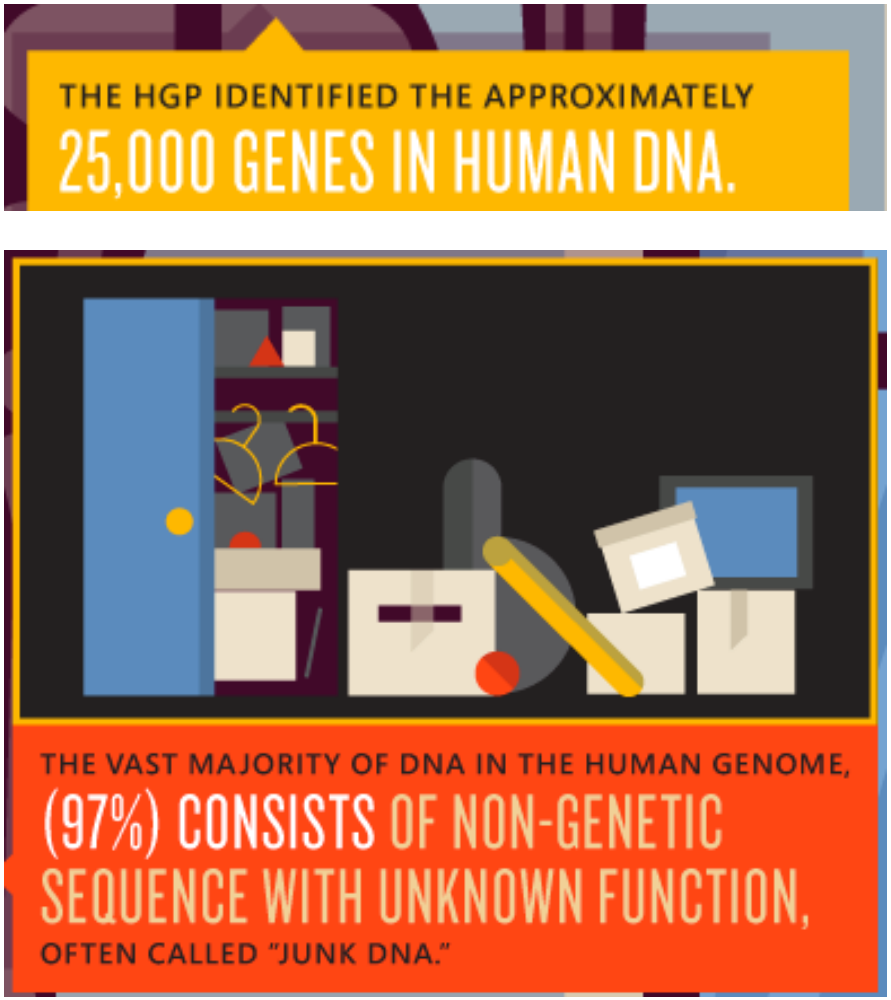
The Human Genome Project



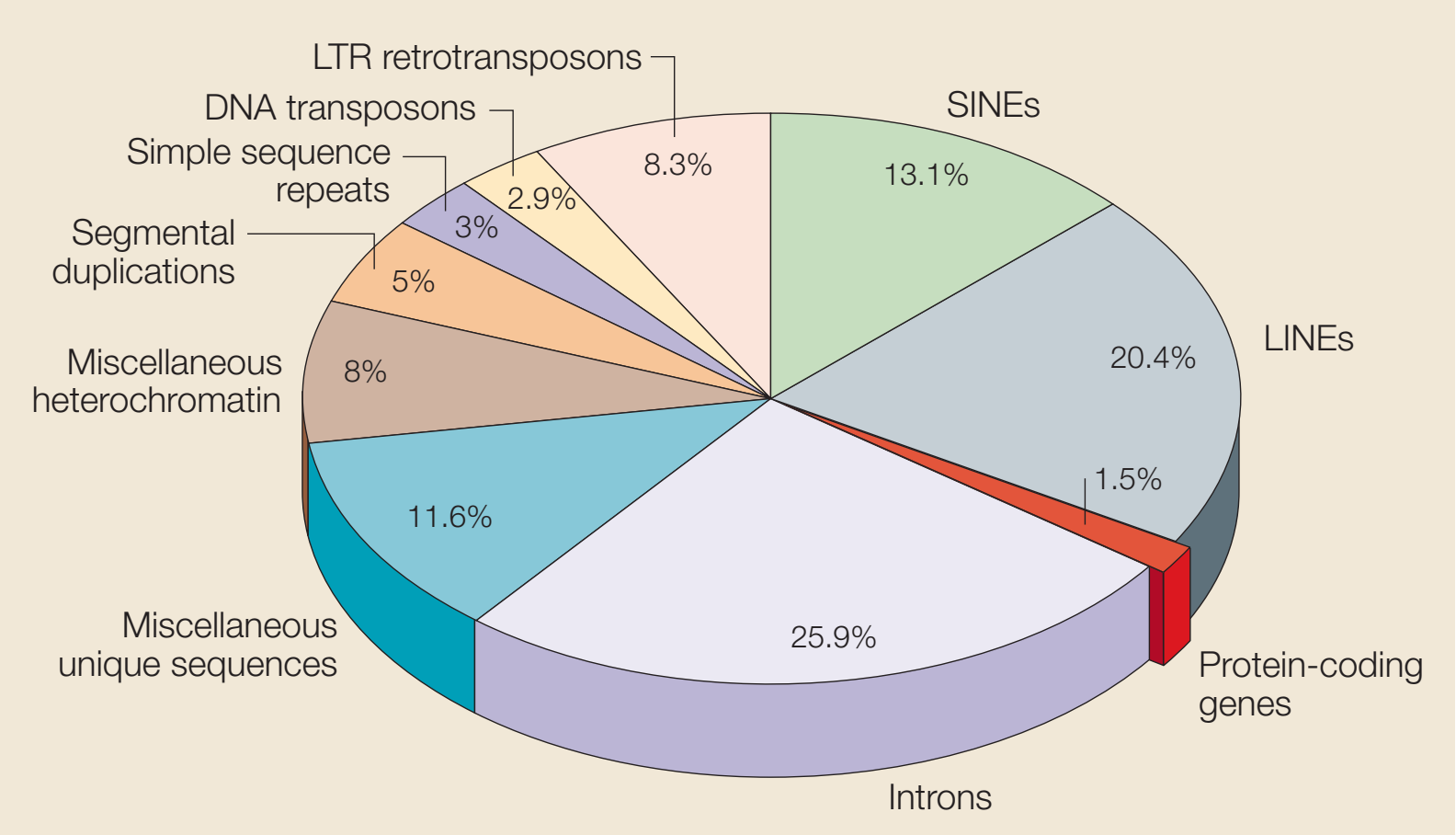
The Human Genome Project



The Human Genome Project



Main components of the eukaryotic genome



GENOMIC VARIATION

Genomic variation

- Single nucleotide polymorphisms (SNPs)
- Insertions/deletions
- Copy number variations (large)
- Variable (short) number tandem repeats

Single nucleotide polymorphisms (SNPs)

A single nucleotide (A,T,C,G) DNA sequence alteration

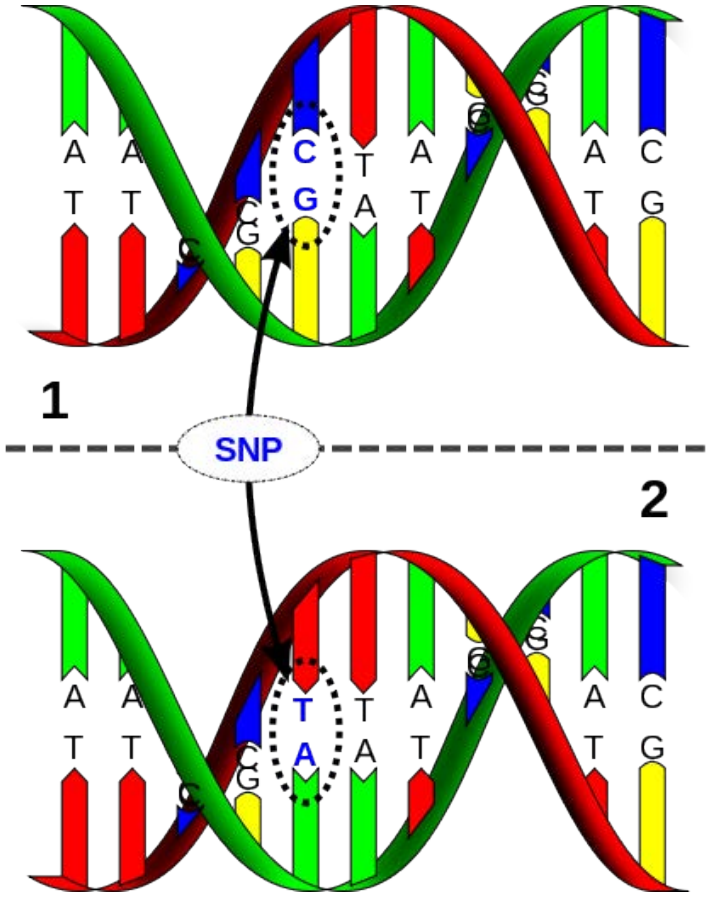
… ACGGCTAA …

… ATGGCTAA …

C and T are the **alleles** for this position

DNA is double stranded

- "C" or "T" on red strand
- "G" or "A" on green strand

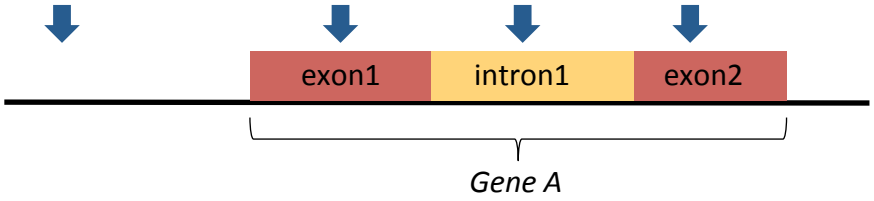


Single nucleotide polymorphisms (SNPs)

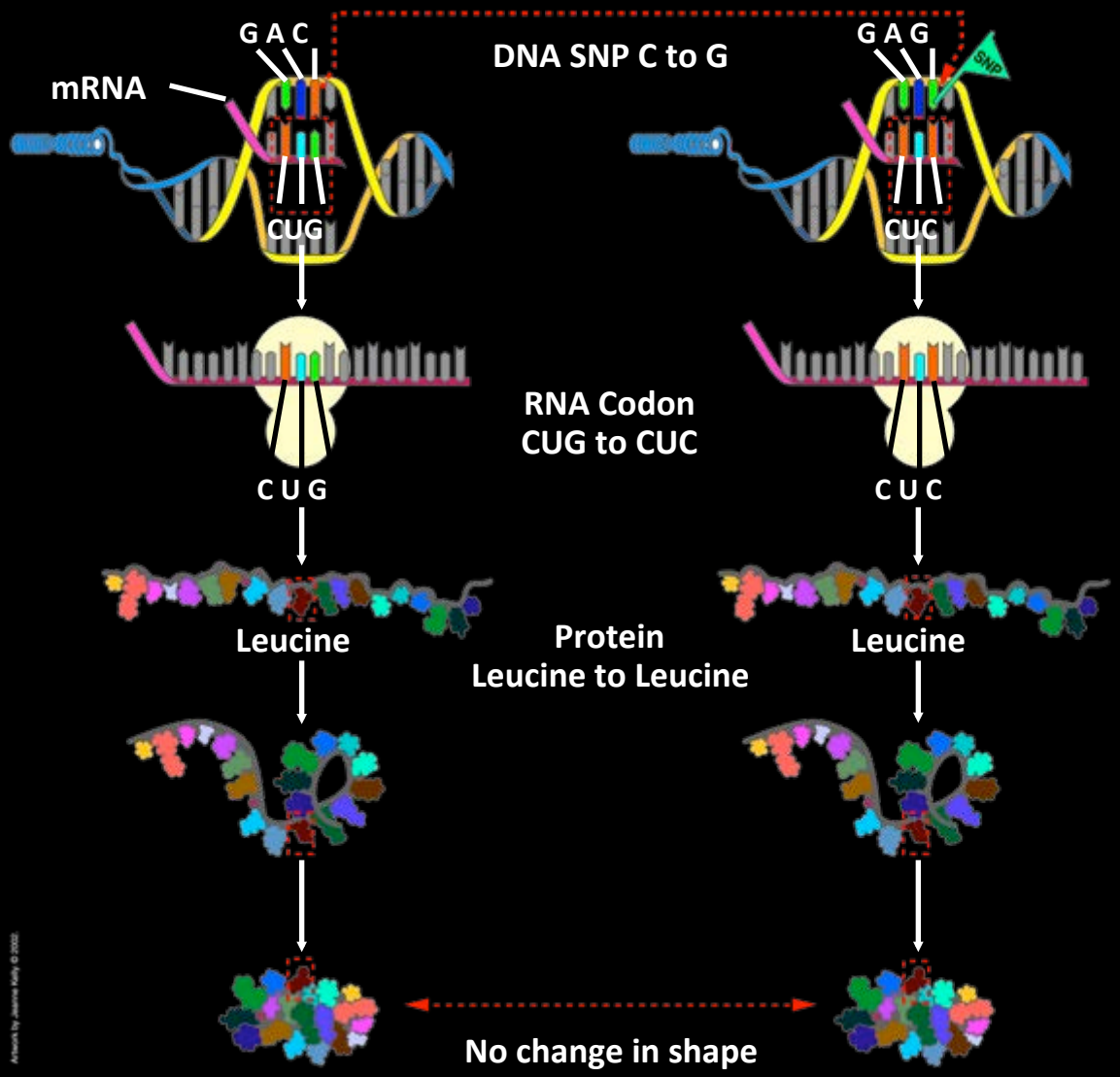
- Occur in at least 1% of the population
- Most common kind of human genetic variation
- 10-30 million SNPs in the human genome
- Occur every 100-300 bases along the 3-billion-base human genome
- Evolutionary stable

Single nucleotide polymorphisms (SNPs)

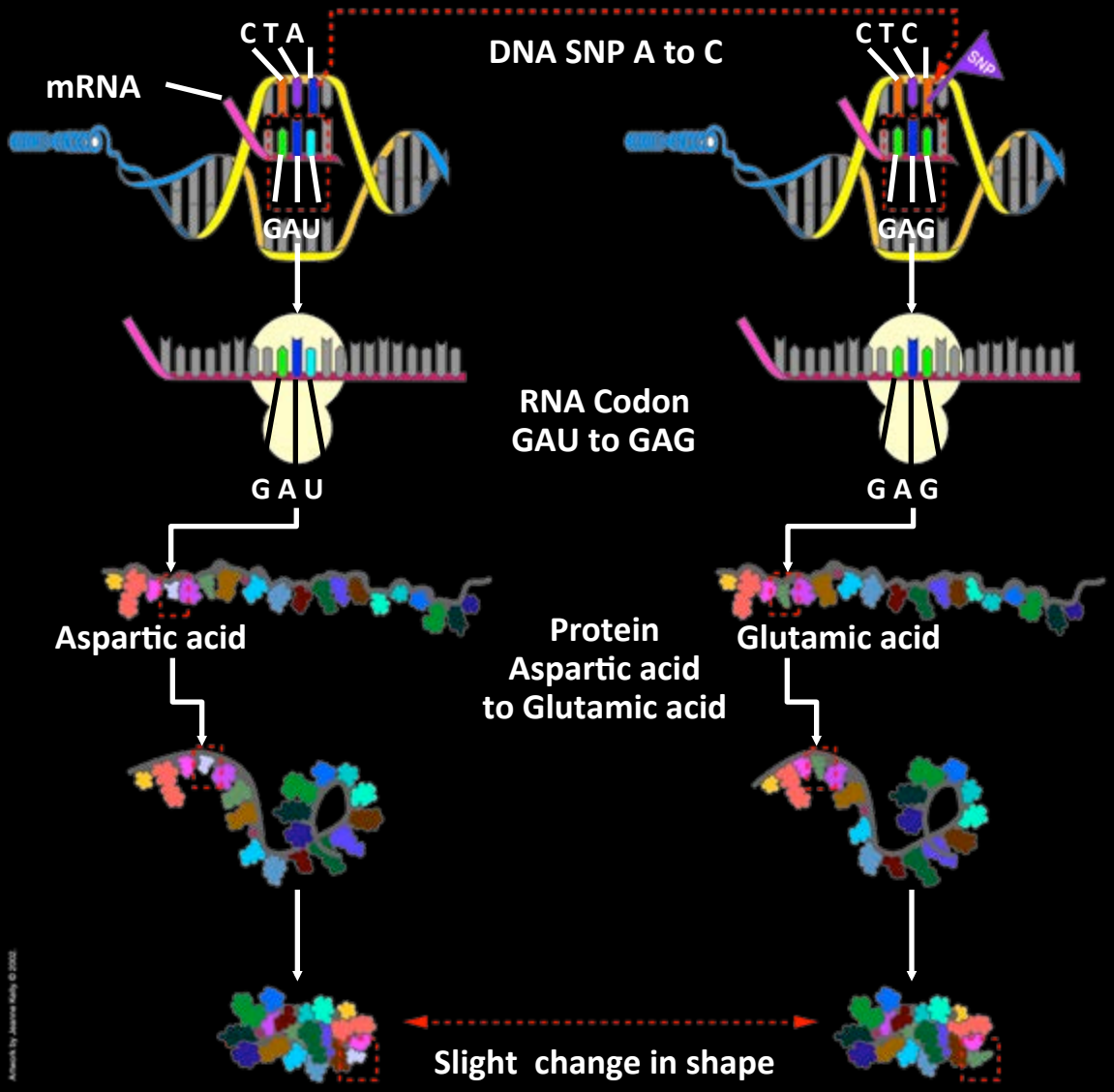
- Non-coding region
- Coding region
  - Synonymous
  - Nonsynonymous



SNPs in Coding Regions – Synonymous: No Changes in Protein

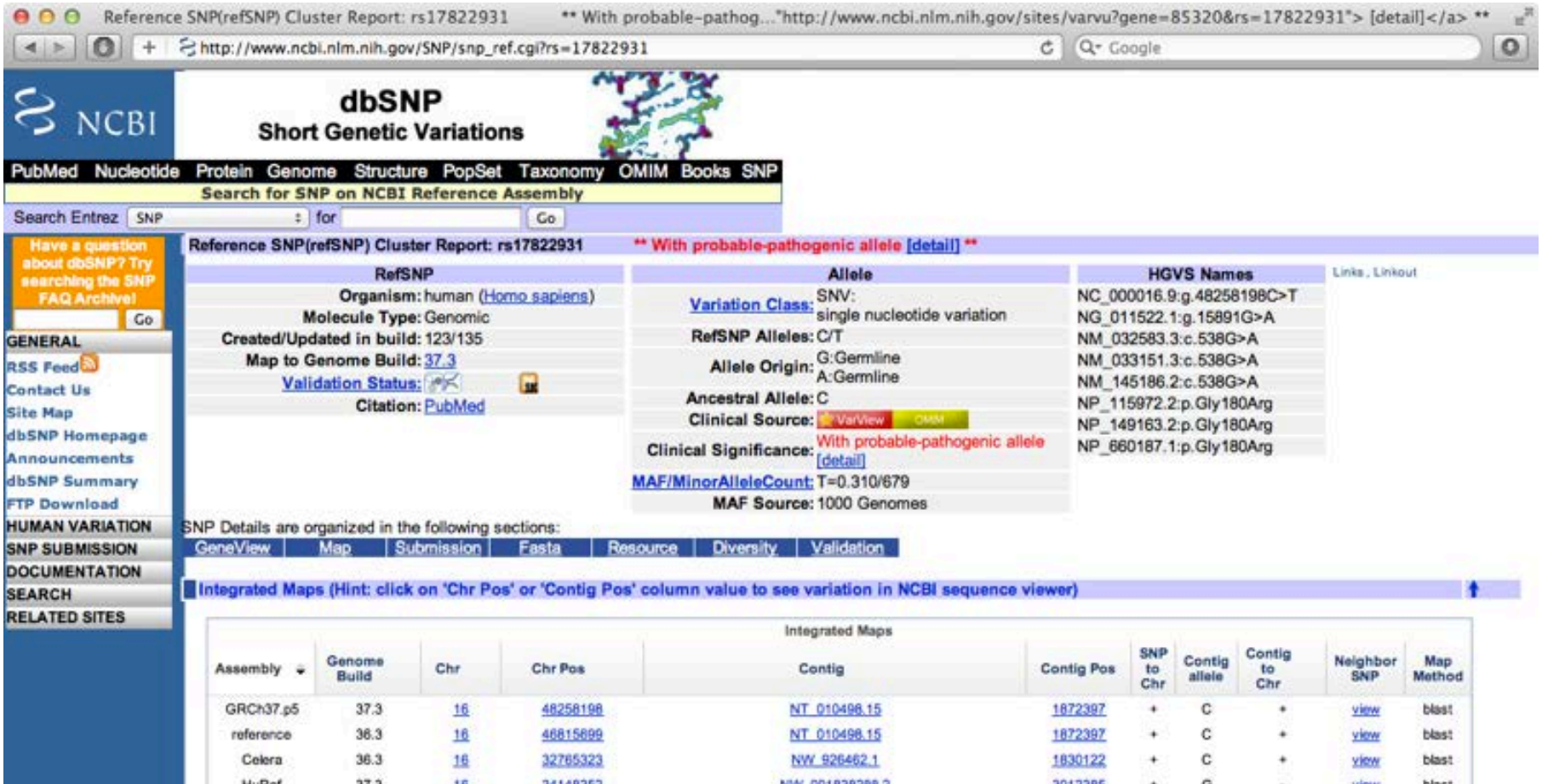


SNPs in Coding Regions – Nonsynonymous: Changes in Protein

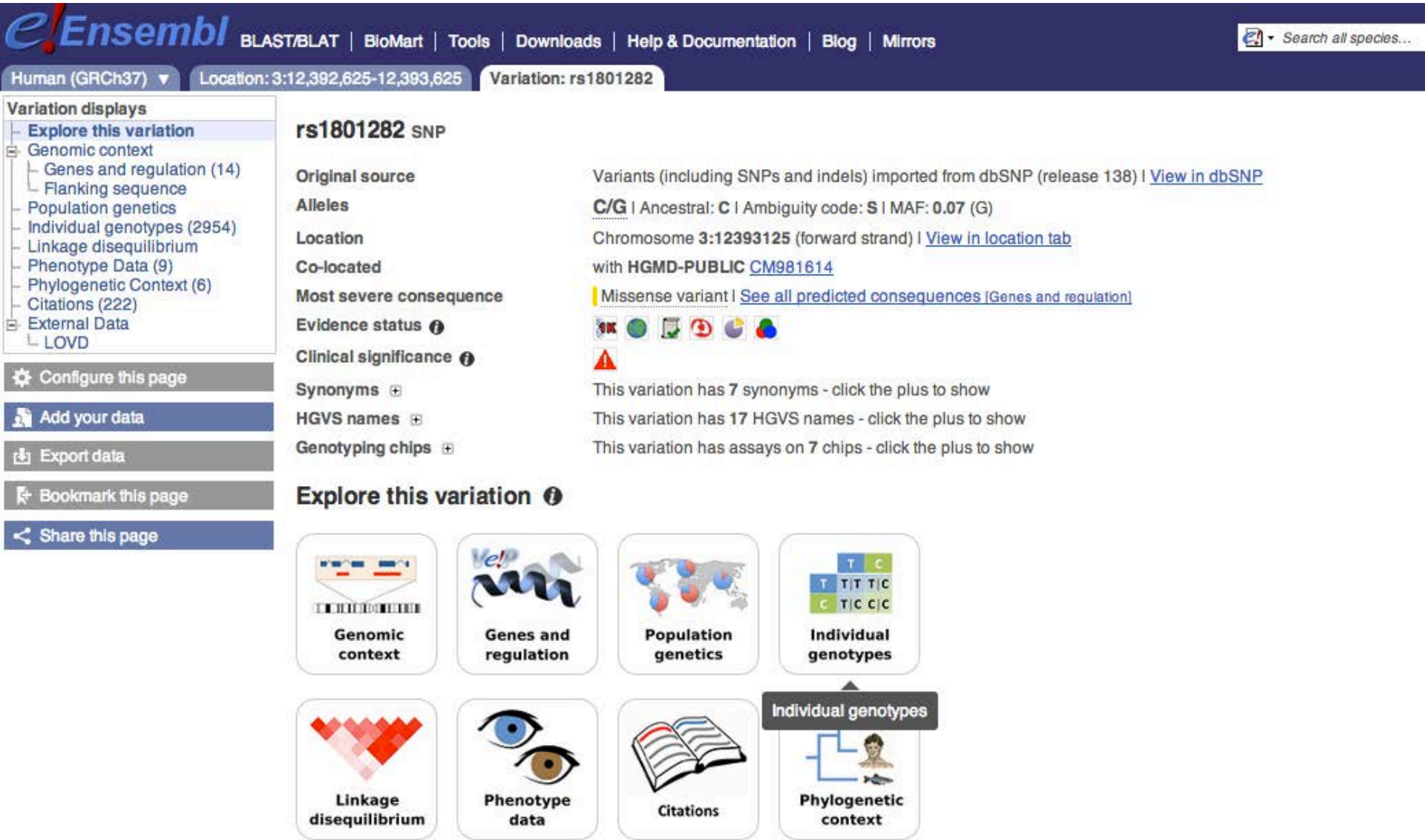


dbSNP database

- rs numbers
- chromosome and positions
- Strand orientation



Ensembl database



GENOTYPES

One copy of rs17822931 from the father and one copy from the mother

Copy 1:

```
5′...GGCCTGAGT...3′  (+)
3′...CCGGACTCA...5′  (-)
```

Copy 2:

```
5′...GGCCCGAGT...3′  (+)
3′...CCGGGCTCA...5′  (-)
```

Genotype for rs17822931 on plus strand:

```
                T;C (T;C)          C,T   T,C   (T,C)
     (C;T)  CT      C;T   rs17822931(T;C)        TC
rs17822931(C;T)
```

Genotype for rs17822931 on minus strand:

```
                A;G (A;G)          C,T   A,G   (A,G)
     (G;A)  GA      G;A   rs17822931(A;G)        AG
rs17822931(G;A)
```

exercise

rs4788084 rs17822931 rs73546424

Copy 1:

5′...TCCCCTGGG...GGCCTGAGT...TGCATGTGA... 3′ (+)

3′...AGGGGACCC...CCGGACTCA...ACGTACACT... 5′ (−)

Copy 2:

5′...TCCCCTGGG...GGCCCGAGT...TGCAAGTGA... 3′ (+)

3′...AGGGGACCC...CCGGGCTCA...ACGTTCACT... 5′ (−)

	rs4788084 dbSNP orientation: minus	rs17822931 dbSNP orientation: plus	rs73546424 dbSNP orientation: plus
genotype on “plus strand”			
genotype on “minus strand”			
genotype on “dbSNP strand”			

HOMOZYGOTES AND HETEROZYGOTES

Homozygous:

Genotype consisting of two identical alleles at a given locus
(For a SNP: the same base at both copies, eg. C;C or A;A)

Heterozygous:

Genotype consisting of two different alleles at a locus
(For a SNP: the same base at both copies, eg. A;C)

exercise

	rs4788084	rs17822931	rs73546424		
Copy 1:	**5′...TCCCCTGGG...GGCCTGAGT...TGCATGTGA...**			**3′**	**(+)**
	3′...AGGGGACCC...CCGGACTCA...ACGTACACT...			5′	(−)
Copy 2:	**5′...TCCCCTGGG...GGCCCGAGT...TGCAAGTGA...**			**3′**	**(+)**
	3′...AGGGGACCC...CCGGGCTCA...ACGTTCACT...			5′	(−)

heterozygous or homozygous ?

heterozygous or homozygous ?

heterozygous or homozygous ?

GENOTYPING

SNP arrays

- Microarray technique
- 0.3 – 4.3 million SNPs

affymetrix
Biology for a better world

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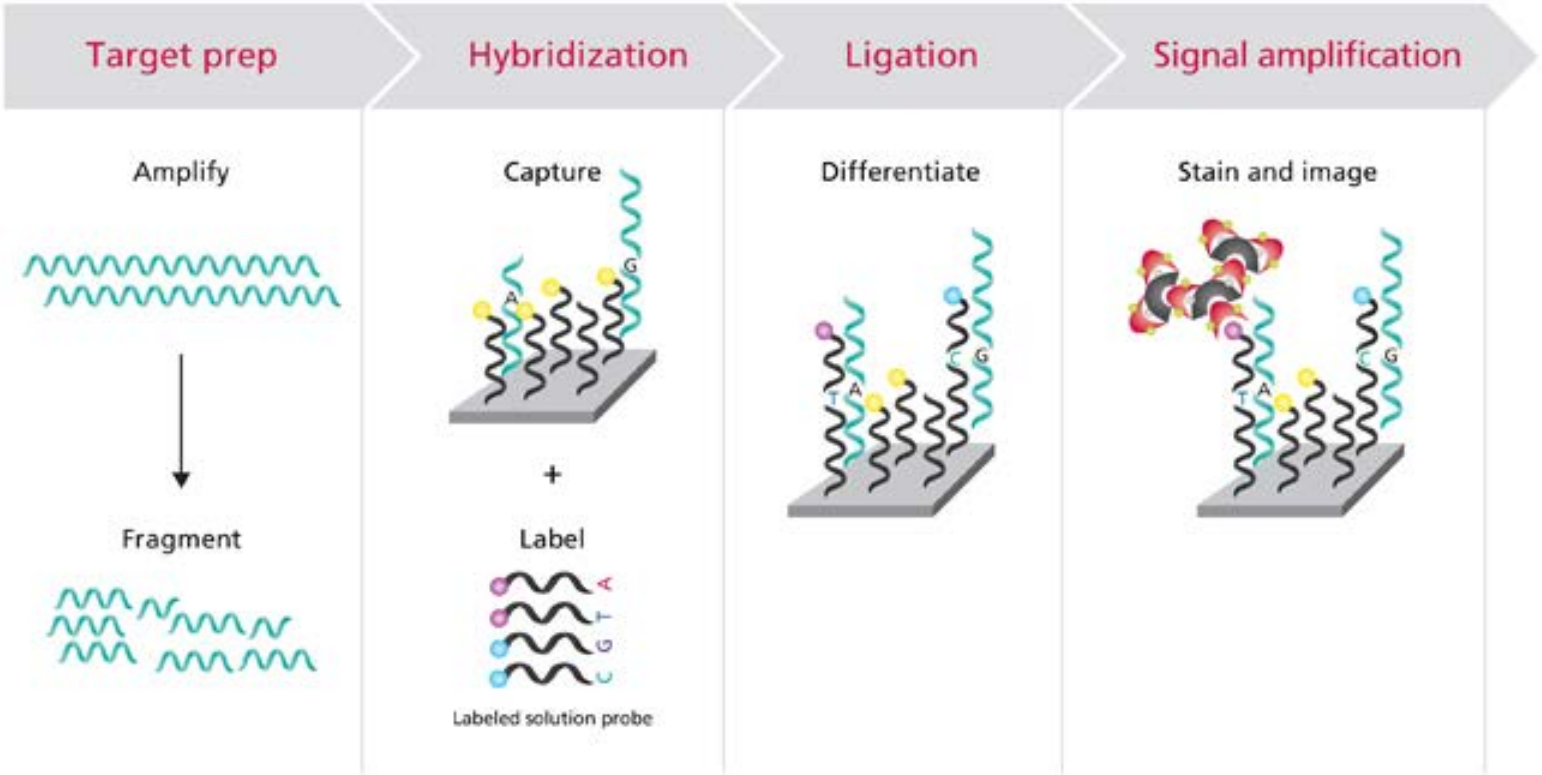

Genomic enlightenment
Medicinsk Museion

Next Generation Sequencing

- Different parts of the genome can be sequenced:
  - ✧ Whole genome
  - ✧ Exome
  - ✧ Targeted
- Different methods for different platforms
- Is increasing in popularity due to increasingly lower costs

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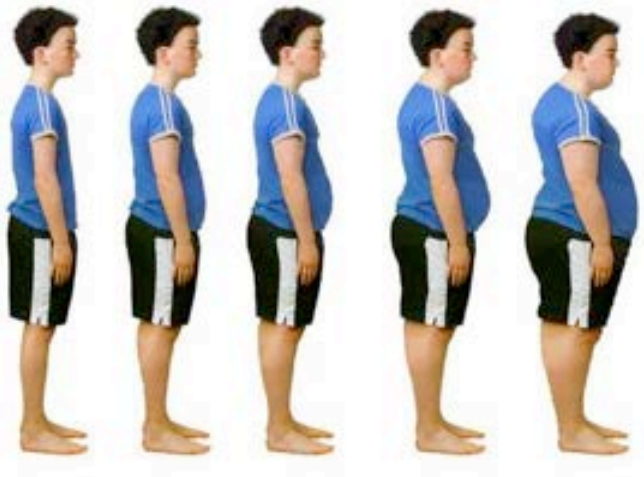
PHENOTYPES

An observable characteristics or trait



obesity

eye color

height

Connecting genotypes and phenotypes:

GENOME-WIDE ASSOCIATION STUDIES (GWAS)

Monogenic and polygenic traits

- Some traits are determined by a **single gene**, where a one mutation can cause a disease
  - Often called Mendelian diseases
  - Examples are Huntington's disease and sickle cell anemia
- Most common traits and diseases are caused by **a large number of genes**
  - Often called complex traits/diseases
  - Examples are human height, obesity, type 2 diabetes and cardiovascular disease
- **GWAS studies usually focus on complex polygenic traits**

GWAS

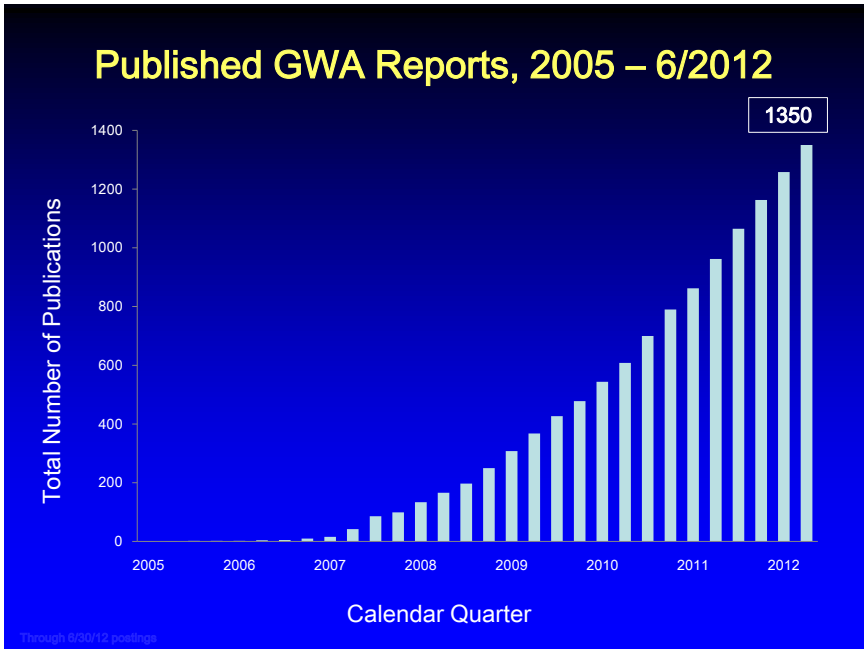
Association of common variants (SNPs) across the whole genome with a particular phenotype

Science 2007:

BREAKTHROUGH OF THE YEAR

Human Genetic Variation

Equipped with faster, cheaper technologies for sequencing DNA and assessing variation in genomes on scales ranging from one to millions of bases, researchers are finding out how truly different we are from one another

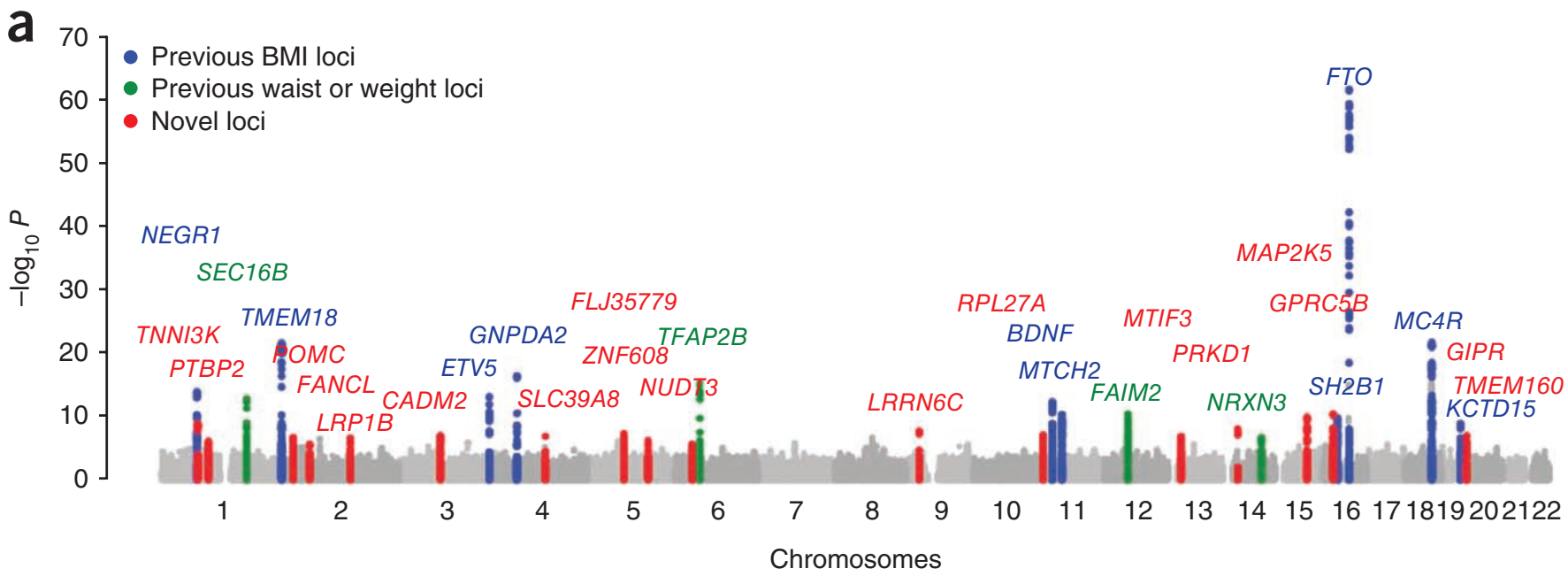


Cases vs controls

- Obtain DNA from a disease group (e.g. asthma) and a control group
- Obtain genotypes
- Identify variants that are significantly more common among cases than controls
- Those SNPs are associated with the disease (in this study)
- Not necessarily causal

Example of GWAS results (BMI)

Manhattan plot displays all SNPs on x-axis (ordered by chromosome) and $-\log_{10}$ of p-values on y-axis

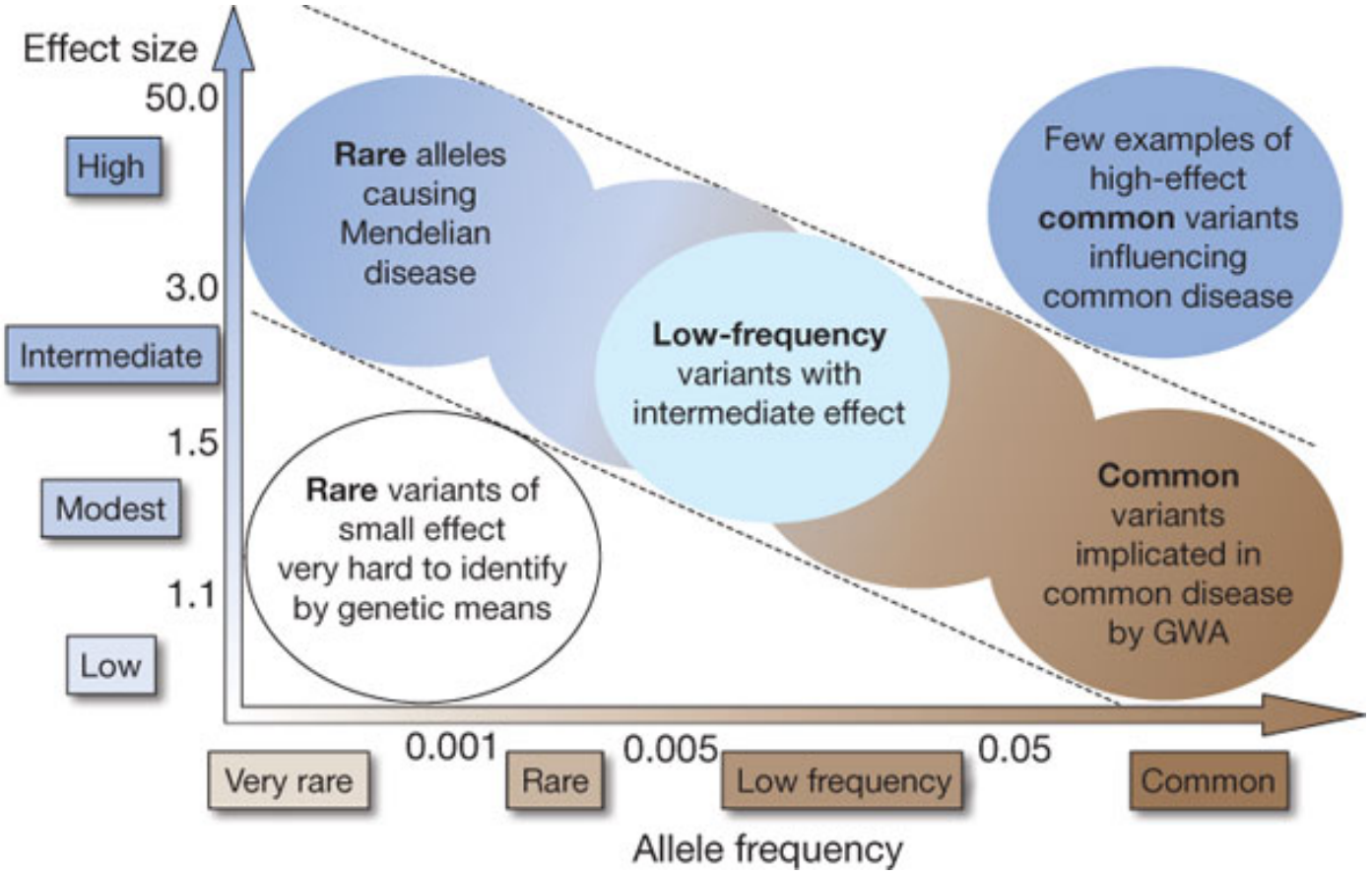


nature genetics

Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index

Speliotes et al, Nature Genetics, 2010

Feasibility of identifying genetic variants by risk allele frequency and strength of genetic effect (odds ratio).

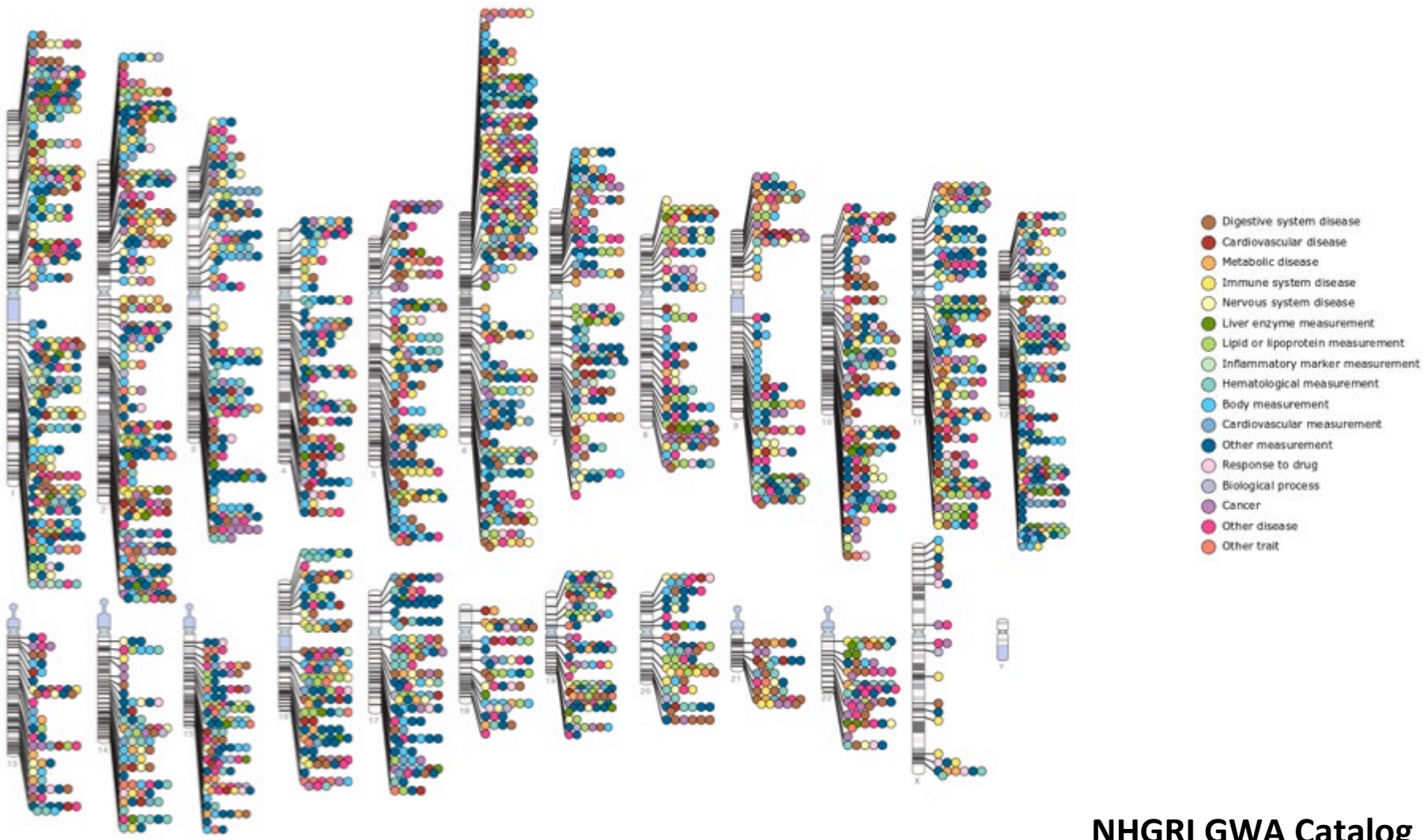


nature

TA Manolio *et al. Nature* **461**, 747-753 (2009) doi:10.1038/nature08494

NHGRI catalog

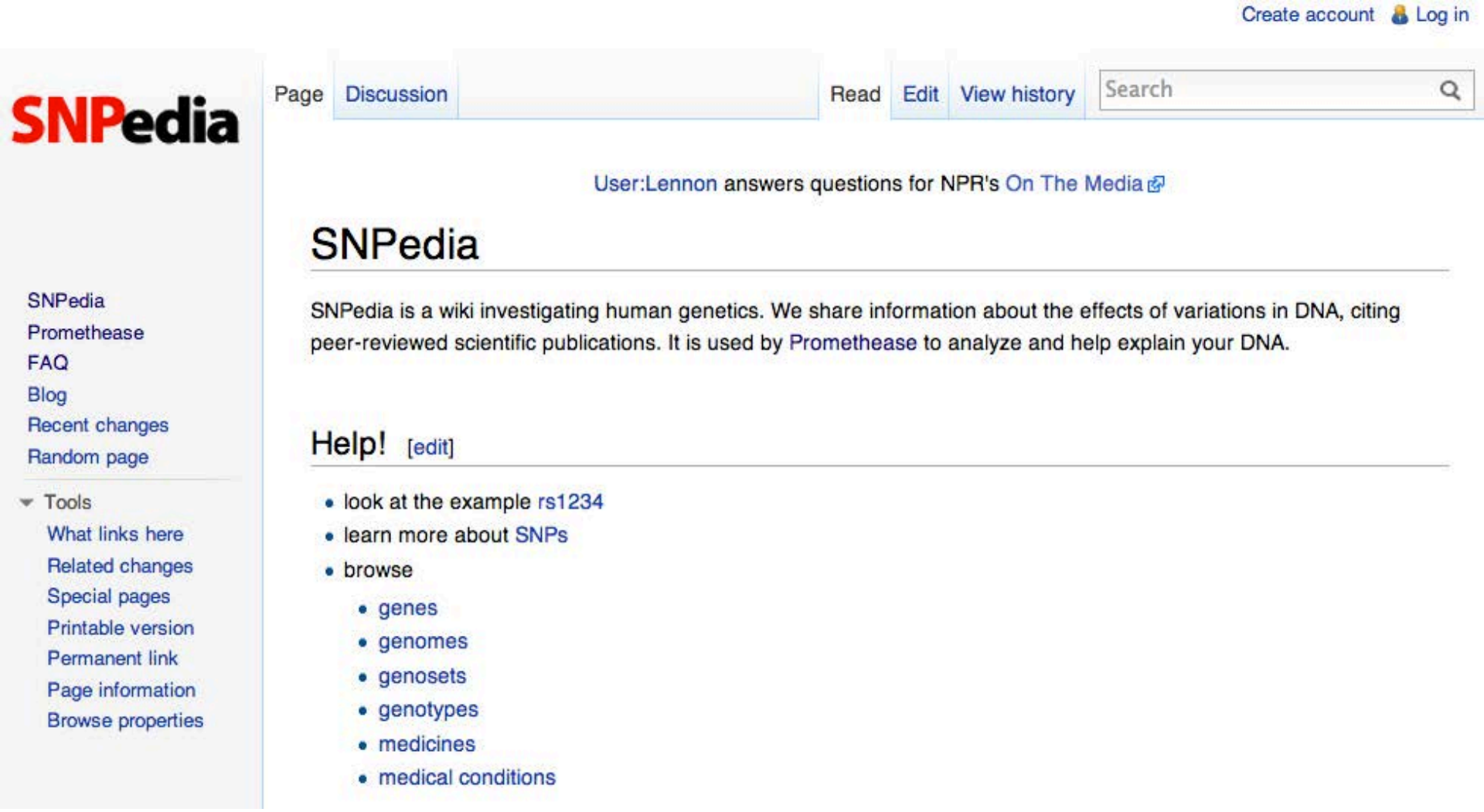
As of 07/01/14, the catalog includes 1782 publications and 12151 SNPs.



National Human Genome Research Institute

NHGRI GWA Catalog
www.genome.gov/GWAStudies

SNPedia.com


Create account
Log in
SNPedia
Page
Discussion
Read
Edit
View history
Search
User:Lennon answers questions for NPR's On The Media
SNPedia
SNPedia is a wiki investigating human genetics. We share information about the effects of variations in DNA, citing peer-reviewed scientific publications. It is used by Promethease to analyze and help explain your DNA.
Help! [edit]
look at the example rs1234
learn more about SNPs
browse
genes
genomes
genosets
genotypes
medicines
medical conditions
SNPedia
Promethease
FAQ
Blog
Recent changes
Random page
Tools
What links here
Related changes
Special pages
Printable version
Permanent link
Page information
Browse properties

Challenges of GWAS

- Missing heritability
- Small effect sizes (OR < 1.5)
- Not much translation into clinical practice
- Biological role of variants unclear, majority (93%) outside of coding regions

The case of the missing heritability

When scientists opened up the human genome, they expected to find the genetic components of common traits and diseases. But they were nowhere to be seen. **Brendan Maher** shines a light on six places where the missing loot could be stashed away.

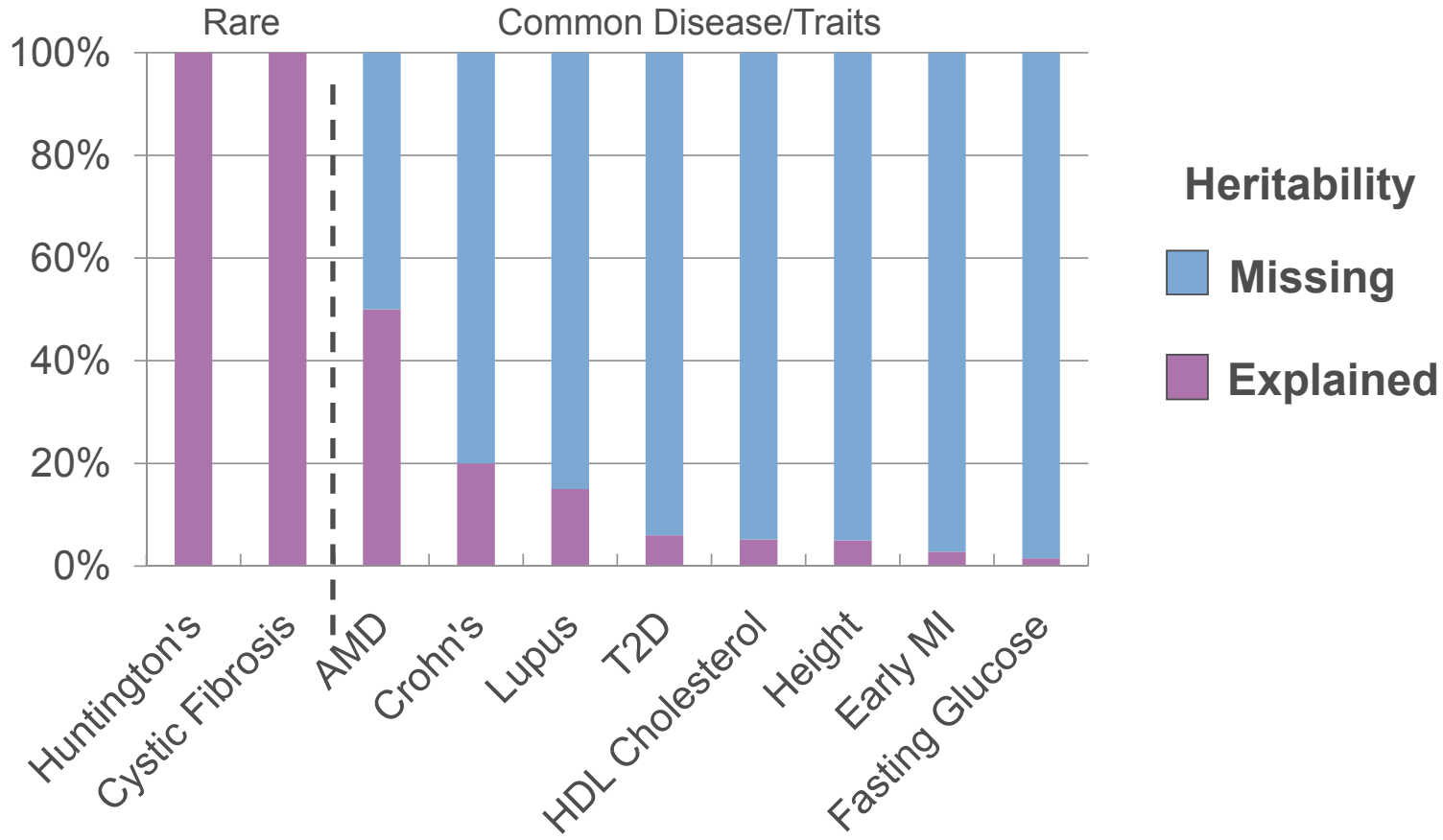
REVIEW

Five Years of GWAS Discovery

Peter M. Visscher,[1,2,*] Matthew A. Brown,[1] Mark I. McCarthy,[3,4] and Jian Yang[5]

The Case of the Missing Heritability

For most common diseases, the sum of individual effects found so far is much less than the total estimated heritability



Adapted from Manolio et al 2009

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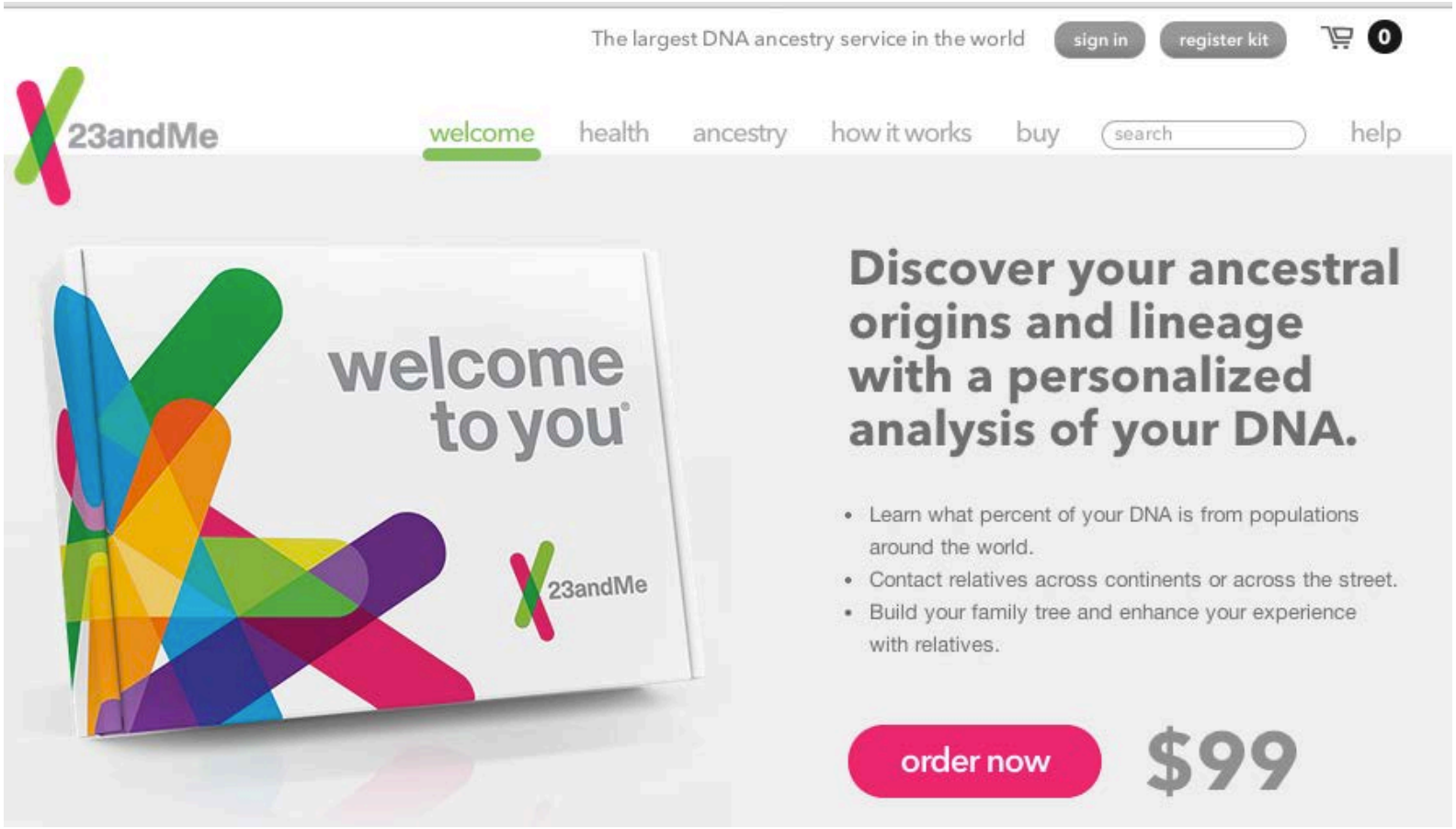
GENOTYPE TO PHENOTYPE PREDICTIONS

It is difficult, perhaps impossible, to accurately predict <u>complex</u> traits from the information we have today!

- Clinical setting
  - High penetrance, often rare mutations, such as *BRCA1*
- Commercial genotyping
  - 23andMe
- Ancient genomes

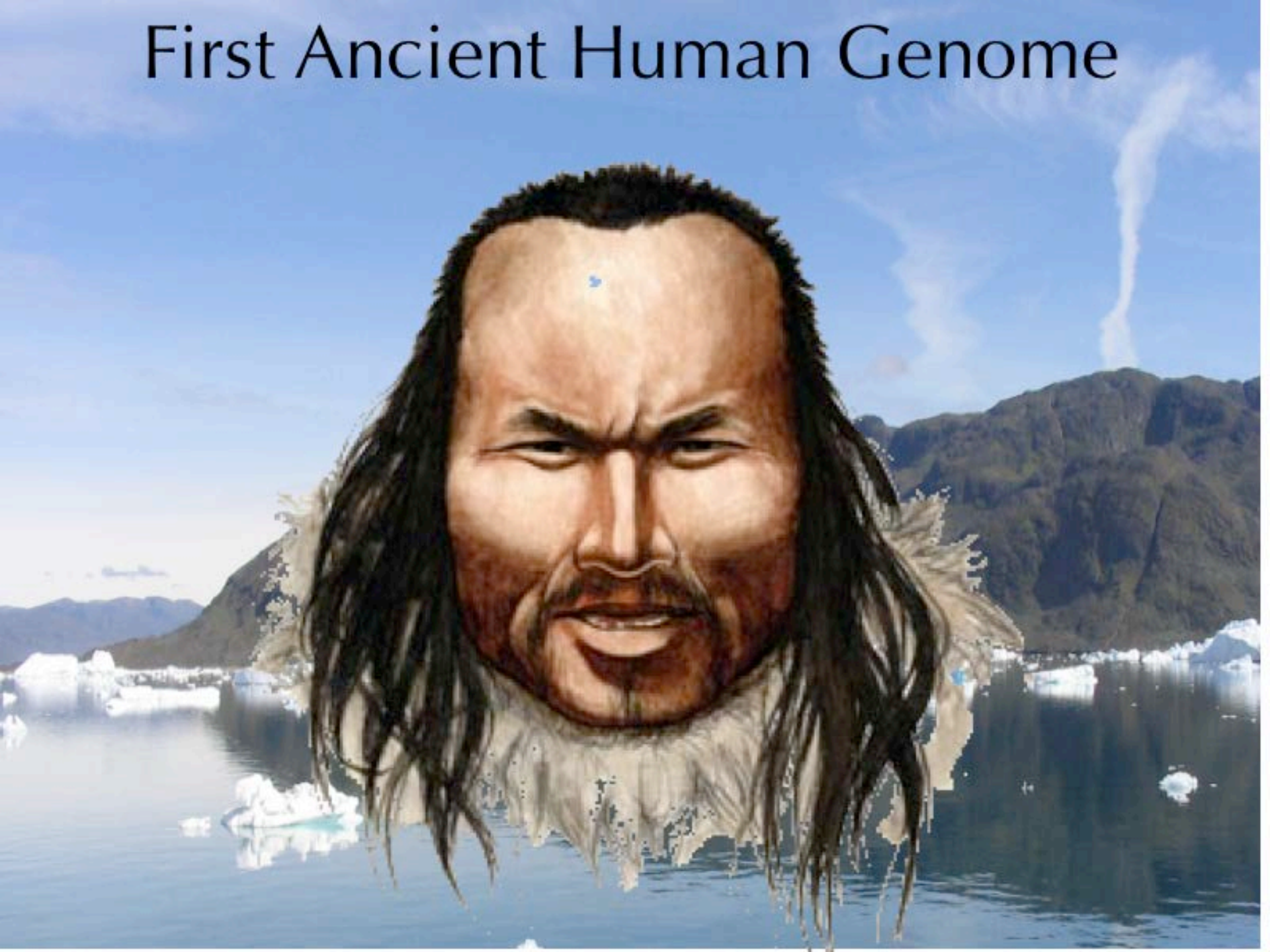
23andMe

Commercial genotyping



Genotype and phenotype of an ancient genome

First Ancient Human Genome

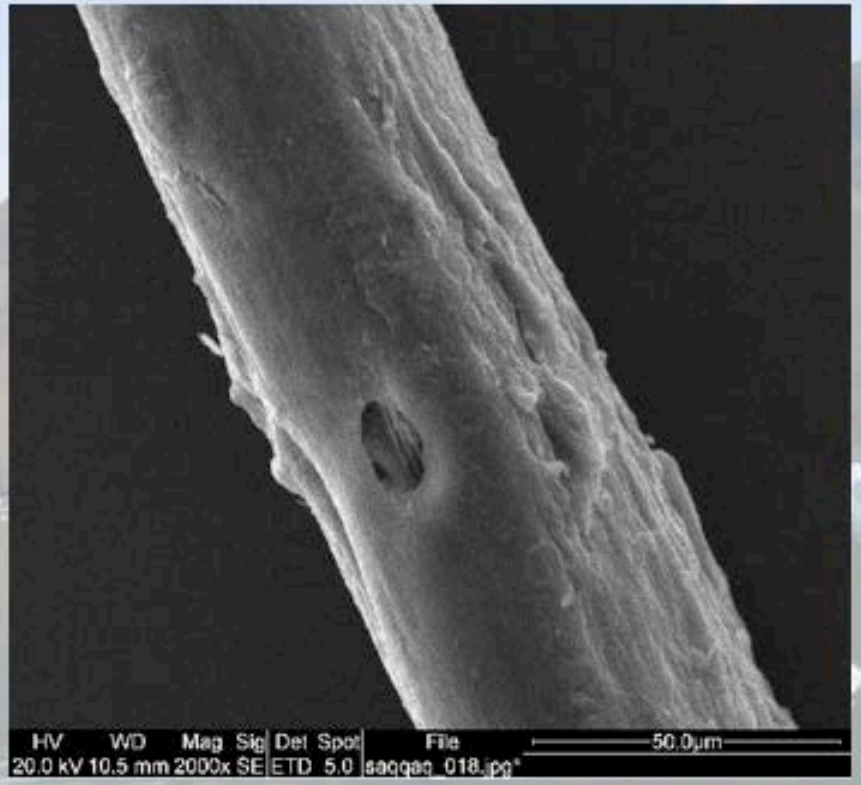


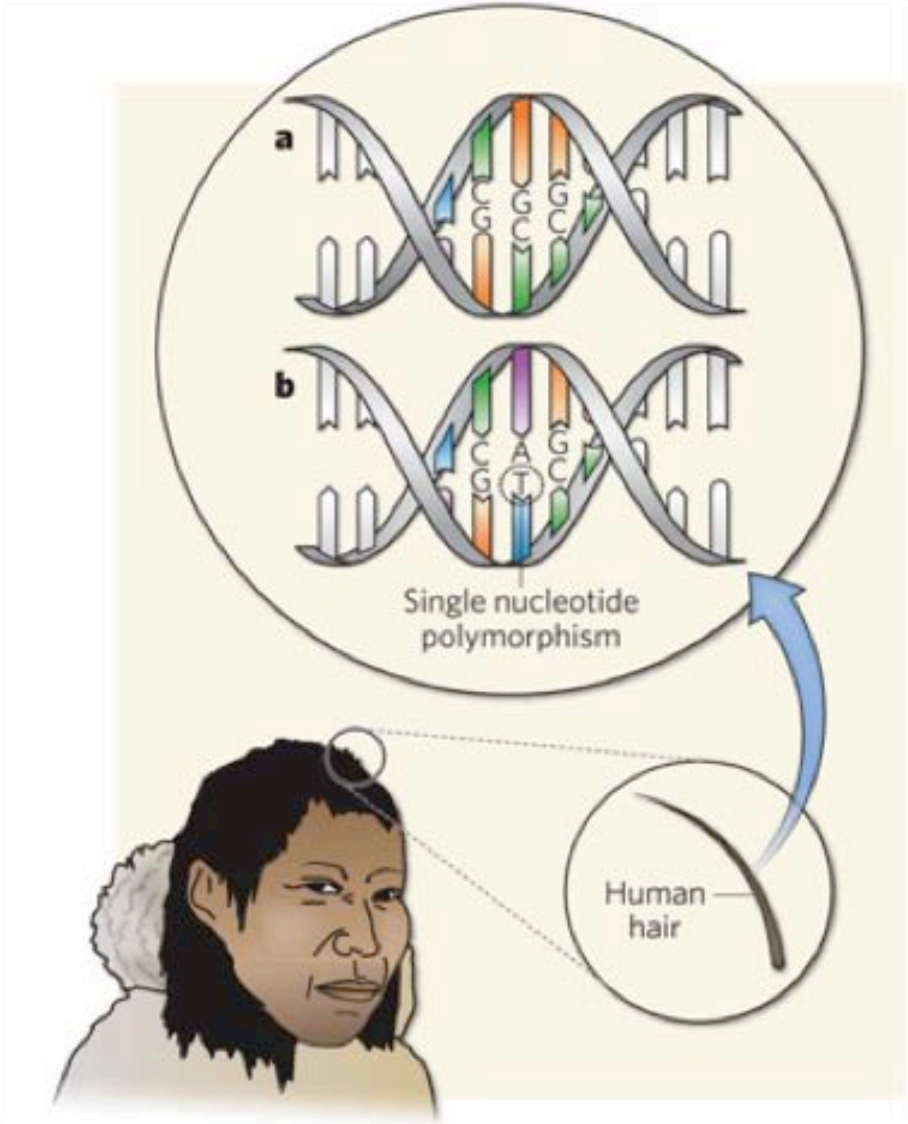
The Saqqaq Genome Project

4,000 years
Hair sample from permafrost
DNA extraction <10% contamination
20 x coverage
Started 2009

Eske Willerslev

DNA from hair

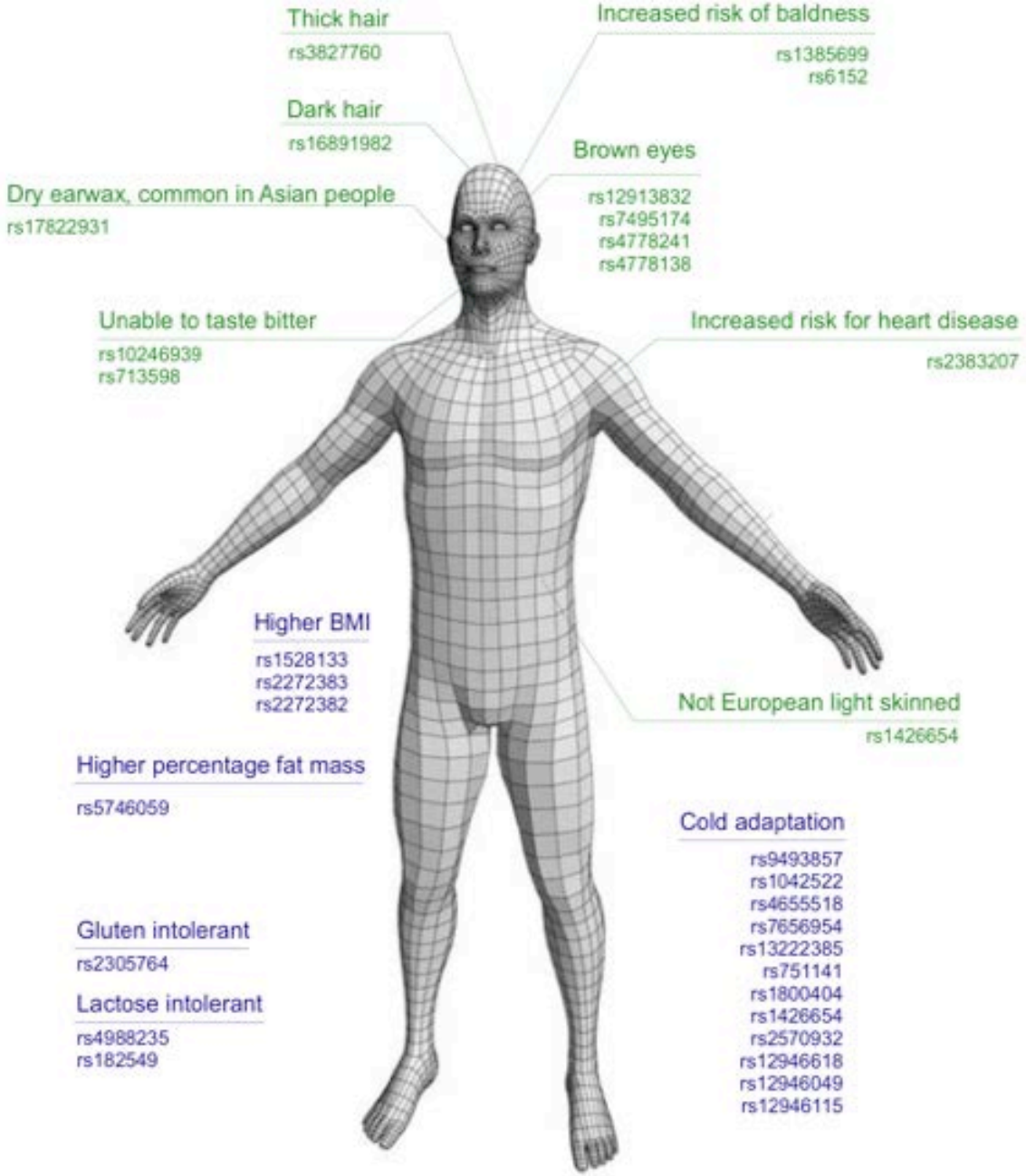




Rasmussen *et al.*[2] have sequenced the genome of a man from the Saqqaq culture, using DNA from hair preserved in permafrost in Greenland. They analysed the genome to find single nucleotide polymorphisms (SNPs) — differences in single DNA base pairs that exist between individual genomes, and that may act as markers of an individual's physical traits. **a**, Here, a short stretch of human DNA is shown that is a marker for normal earwax. **b**, In the analogous DNA from the Saqqaq individual, there is a SNP in which a C in the lower strand has been replaced by a T (C, G, T and A denote the four kinds of DNA base). This SNP shows that the Saqqaq man had dry earwax. Rasmussen and colleagues identified other SNPs indicating that the ancient human had, among other things, brown eyes, non-white skin, thick dark hair and an increased susceptibility to baldness.

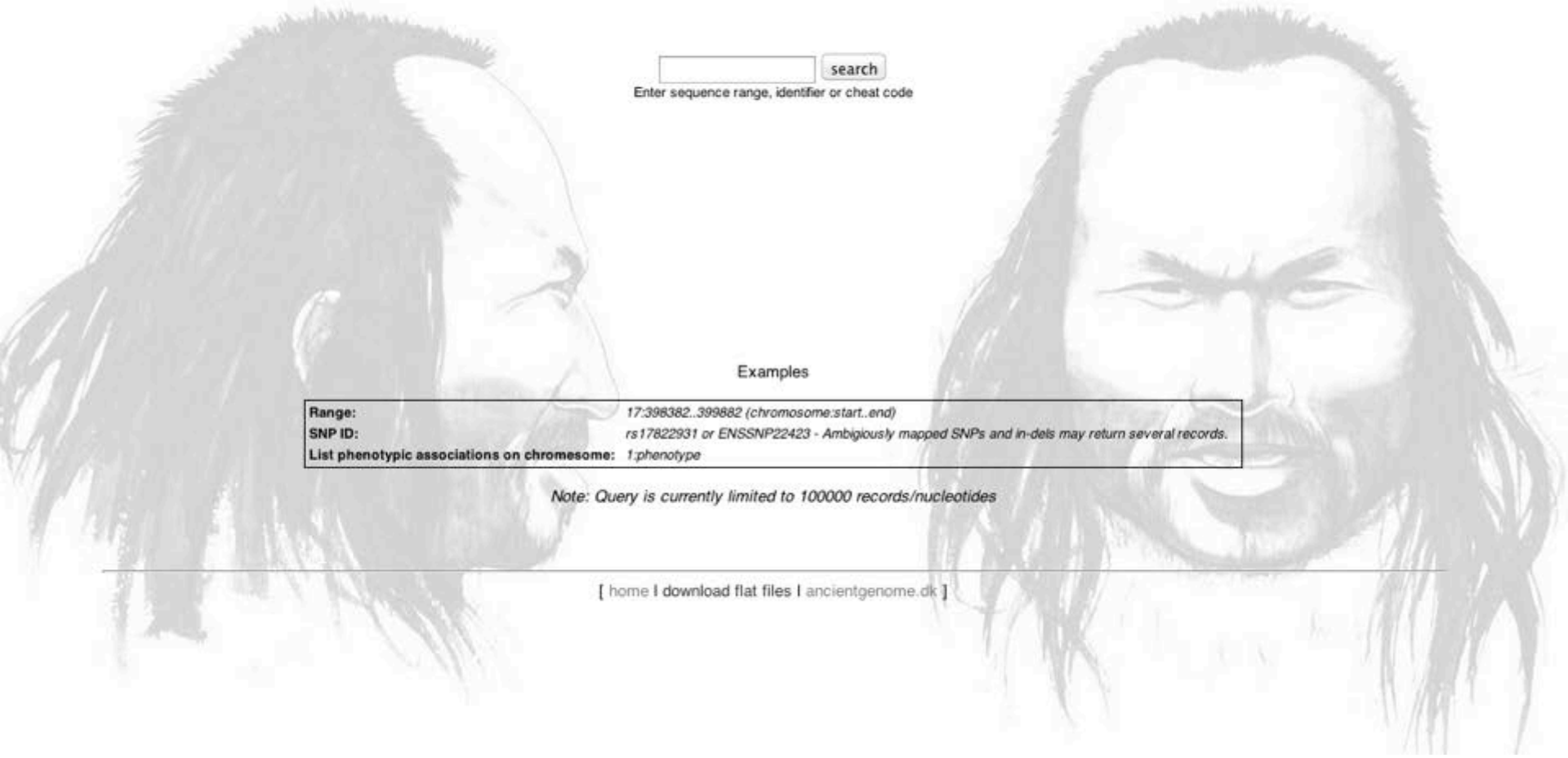
V. WARD, UNIV. AUCKLAND

What can we say about his phenotypes?



From genotype to phenotype: how good are we at putting a face to an anonymous individual? - While some traits manifest themselves in a tissue specific manner (highlighted in green), others are more systemic (highlighted in blue). Going from the genetic blueprint to visual appearance, physiological behaviour and medical predispositions is still an open challenge.

The Saqqaq Genome Database (NCBI36)



search

Enter sequence range, identifier or cheat code

Examples

Range:	*17:398382..399882 (chromosome:start..end)*
SNP ID:	*rs17822931 or ENSSNP22423 - Ambigiously mapped SNPs and in-dels may return several records.*
List phenotypic associations on chromesome:	*1:phenotype*

Note: Query is currently limited to 100000 records/nucleotides

The Saqqaq Genome Database (NCBI36)

Result

chr	pos	ref	is_ref	genotype	pp	depth	repeat	rs	type	strand	snp_alleles	trait	risk_allele	pmid	source
3	12368125	C	n	GG	0.0006439	11		rs1801282	single	+	CG	obesity, Obesity, association with, Disease-associated and putatively functional polymorphism,Type 2 diabetes	C	9792554 17463246 17463248 17463249	NHGRI,other

Columns explained

chr	The chromosome
pos	Position on chromosome
ref	The reference nucleotide on forward strand in hg18
is_ref	Indicates whether the genotype is the same as the reference nucleotide (y) or not (n)
genotype	The genotype called (on forward strand)
pp	For numerical reasons, we report (1-PP), where PP is the posterior probability of the genotype
depth	The number of reads covering the position
repeat	If the position lies in an annotated repeat, the ID is given here
rs	dbSNP rs-number
type	Type of dbSNP entry ("single", "indel" etc. - see the UCSC genome browser for details).
strand	Strand for dbSNP entry + (or 1) or - (or -1)
snp_alleles	Known SNP alleles, e.g. "AC" (or "A/C") for a SNP of type "single"
trait	Associated trait or phenotype (general information -- not dependant on this individual's genotype)
risk_allele	Allele associated with trait
pmid	Pubmed ID of GWAS paper reporting the association
source	Source database for association

EXERCISE

Genotype to phenotype exercise

http://wiki.cbs.dtu.dk/teachingmaterials/index.php/ExGenotype2PhenotypeLite